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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Fowlkes et al.	Confirmation No.: 4623
Serial No.: 09/286,166	Group Art Unit: 1646
Filed: April 5, 1999	Examiner: Michael T. Brannock
For: YEAST CELLS ENGINEERED TO PRODUCE PHEROMONE SYSTEM PROTEIN SURROGATES, AND USES THEREFOR	Atty Docket No.: 11072-009-999 (formerly CPI-012CP4BCN)

DECLARATION OF DR. JAMES BROACH UNDER 37 C.F.R. §1.132

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Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Dr. James Broach, Ph.D., do declare and state that:

1. I am currently Professor of Molecular Biology and Associate Director of Lewis-Sigler Institute for Integrative Genomics at Princeton University in Princeton, NJ. My academic and technical experience and honors, and a list of my publications, are set forth in my *curriculum vitae*, attached hereto as Appendix 1.

2. I am a co-inventor of the invention described and claimed in the above-identified U.S. Application No. 09/286,166 ("the '166 application"). I have read and understand the '166 application, and have been asked to comment on its teachings, in particular, with regard to the enablement of the claimed invention, which encompasses a yeast cell comprising (i) a reporter gene under control of a pheromone-responsive promoter, (ii) a heterologous G protein-coupled receptor ("GPCR") gene under the control of a separate promoter, (iii) a mutation in the Gα subunit gene (a SCG1/GPA gene), and (iv) a hybrid Gα protein, wherein said heterologous GPCR gene does not include a coding sequence from a yeast GPCR gene.

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3. The following experiments were carried out by me or under my supervision. These experiments demonstrate the construction of a number of mammalian G protein-coupled receptor genes that do not include a coding sequence from a yeast G protein-coupled receptor gene, the expression of such constructs in a yeast cell, and the successful application of such constructs for use in the screening methods described in the '166 application.

4. Representative human G protein-coupled receptor genes that have successfully been expressed in yeast cells and used in the methods described in the '166 application are provided in Table 1. The first such human G protein-coupled receptor is exemplified as Example 10 at page 115, line 28, to page 120, line 14, of the '166 application. In this example, the human C5a receptor sequence, without any yeast coding sequences, was expressed in a yeast cell with a hybrid Gα protein, and, in the presence of C5a was capable of activating the pheromone response transduction pathway. To this end, the human C5a receptor sequence was linked to a yeast non-coding sequence comprising the constitutive promoter of the highly expressed phosphoglycerate kinase (PGK) gene to construct the human C5a receptor expression vector PGKp-C5aR. Only control non-coding sequences of the PGK gene, and no coding sequences from a yeast GPCR gene or any other yeast gene, are present in this construct.

5. PGKp-C5aR was then tested for activity in yeast by transferring it in a yeast host cell having the following significant modifications: (a) a reporter gene under the control of a pheromone-responsive promoter, *i.e.*, the pheromone-inducible HIS3 gene, FUS1p-HIS3; and (b) a hybrid gene containing sequences encoding the N-terminal portion of the yeast Gα subunit fused to sequences encoding the C-terminal portion of the human Gα subunit which replaces the GPA1 gene at its location on the yeast chromosome, constituting both a mutation in an SCG1/GPA1 gene and a hybrid Gα protein (see specification on page 118, line 29, to page 119, line 9). Activity of the C5a receptor expression construct was tested by autologous expression of C5a, followed by measuring cell growth on media lacking histidine, indicating activation of expression from the HIS reporter gene under the control of the pheromone-responsive promoter, and evidencing

activation of the yeast pheromone response pathway (see specification on page 118, line 29, to page 120, line 14). Accordingly, Example 10 of the instant application enables the yeast cells comprising a heterologous GPCR gene, the C5a receptor gene, wherein said heterologous C5a receptor gene does not include a coding sequence from a yeast GPCR gene.

6. Another example of a human GPCR gene that has been expressed in yeast cells and successfully used in methods described in the '166 application is the human melatonin receptor (ML1aR) gene. First, an expression vector for the ML1aR gene, PGKp-ML1aR, was constructed using the PGK promoter linked to the human melatonin receptor (ML1aR) gene. This construct does not contain coding sequence from a yeast G protein-coupled receptor gene, or any other yeast gene.

7. Next, PGKp-ML1aR was transferred to a yeast strain having the following: (a) reporter gene having LacZ under the control of a pheromone inducible promoter; and (b) a hybrid gene containing sequence encoding the first 41 amino acids of the yeast Gα subunit fused to sequences encoding human Gαi2, replacing GPA1 at its normal chromosomal location. Addition of ML1aR ligand, *i.e.*, melatonin, resulted in the expression of LacZ from the reporter construct, indicating activation of the yeast pheromone response pathway.

8. A third example takes advantage of the a human GPCR gene, the formyl peptide receptor-1 (FPR1) gene. The FPR1 gene was linked to the PGK promoter to construct the PGKp-FPR1, which does not contain any coding sequence from a yeast G protein-coupled receptor gene. PGKp-FPR1 was then expressed in a yeast strain having a pheromone inducible reporter gene FUS1p-HIS3 integrated at the FUS1 locus and a yeast/human hybrid Gα subunit gene replacing GPA1 at its normal chromosomal location. In the presence of the FPR1 ligand, *i.e.*, f-met-leu-phe (fMLP), the yeast pheromone response pathway was activated, as indicated by growth of yeast cells on histidine in the presence of FPR1 ligand.

9. In addition to the human GPCRs described above, ten other examples of human GPCRs which have been constructed, expressed in yeast, and used for the screening assays described in the '166 application, are listed in Table 1. In particular, the human genes for NPY-Y1 and Y2, the human formyl peptide receptor-like 1 (FPRL1), the human chemokine receptor (CXCR4), the heterodimeric GPCR comprising the receptor-activity-modifying protein-1 (RAMP1) and calcitonin gene-related peptide (CGRP), the human neurotensin receptor-2 gene (hNTR), the heterodimeric adrenomedulin receptor comprising the receptor-activity-modifying protein-2 (RAMP2) and CT receptor-like receptor (CRLR), vasopressin, GRP and orexin are each listed in Table 1. Constructs of each of these receptors were expressed in cells containing a hybrid Gα protein, a pheromone-inducible reporter gene, and were found to successfully respond in the presence of ligand, as indicated by the expression of the reporter gene. None of the foregoing receptor constructs contain any coding sequence from a yeast G protein-coupled receptor gene.

10. In summary, we have successfully constructed a series of heterologous G protein-coupled receptors according to the methods of the present application, wherein said heterologous G protein-coupled receptor gene does not include a coding sequence from a yeast G protein-coupled receptor gene. Moreover, such constructs have been demonstrated to be functional in the screening assays in yeast cells as described and enabled by the specification of the present application.

11. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/30/02

James Broach, Ph.D.

Table 1.

Receptor	Construct	Strain	Reporter	G Protein	Background
C5a	PGKp-C5aR	2208	His	gpa1(41)-Gαi2	ste3 ste14
ML1a	PGKp-ML1aR	6536	LacZ	gpa1(41)-Gαi2	ste3 ste14
FPR-1	PGKp-FPR1	9625	LacZ	gpa1(41)-Gαi2	ste3 ste14 ste18-sg6
FPRL1	PGKp-FPRL1	7598	His	gpa1(41)-Gαi2	ste3 ste14
NPY-Y1	PGKp-MFα1prepro-NPY-Y1-FLU-PHO5term	13446	LacZ	GPA1	ste3 ste14 sst2 ste18-sg6
NPY-Y2	PGKp-MFα1prepro-NPY-Y2-FLU-PHO5term	13556		GPA1p-ratGαi1	ste3 ste14 ste18-sg6
CXCR4	PGKp-MFα1prepro-CXCR4-PHO5ter	17732	LacZ	GPA1-Gαi2(5)	sst2 ste3 ste14
CGRP	PGKp-CRLR PGKp-RAMP1	19889	LacZ	GPA1-GαsD229S	ste3 ste18-sg6 stp22
Neurotensin-2	PGKp-MFα1-MFα1prepro-hNTR-PHO5term	13864	LacZ	GPA1-Gα16(6)	
Adrenomedulin	PGKp-CRLR PGKp-RAMP2		LacZ		
Vasopressin	PGKp-MFα1prepro-F-V2R		LacZ	GPA1-Gαs(6)	
GRP	PGKp-MFα1prepro-rGRPR-PHO5t	19910		GPA1-Gαz(5)	ste3 ste14 sst2 ste18-sg6
Orexin	PGKp-MFαF-Orexin2R			Gαq(1-21)-GPA1-Gαq(6)	